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Rubisco expression in rice leaves is related to genotypic variation of photosynthesis under elevated growth CO_2 and temperature

R. W. GESCH[1], I.-H. KANG[2], M. GALLO-MEAGHER[2], J. C. V. VU[3], K. J. BOOTE[2], L. H. ALLEN, J.R.[3] & G. BOWES[4]

[1]USDA-ARS, North Central Soil Conservation Research Laboratory, 803 Iowa Avenue, Morris, MN 56267 USA, [2]Agronomy Department, University of Florida, Gainesville, FL 32611 USA, [3]USDA-ARS, Agronomy, Physiology and Genetics Laboratory, Gainesville, FL 32611 USA and [4]Department of Botany, University of Florida, Gainesville, FL 32611 USA

ABSTRACT

Genetic modifications of agronomic crops will likely be necessary to cope with global climate change. This study tested the hypotheses that genotypic differences in rice (*Oryza sativa* L.) leaf photosynthesis at elevated [CO_2] and temperature are related to protein and gene expression of Rubisco, and that high growth temperatures under elevated [CO_2] negatively affect photosystem II (PSII) photochemical efficiency. Two rice cultivars representing an indica (cv. IR72) and japonica type (cv. M103) were grown in 350 (ambient) and 700 (elevated) μmol CO_2 mol^{-1} at 28/18, 34/24 and 40/30 °C sinusoidal maximum/minimum, day/night temperatures in outdoor, sunlit, environment-controlled chambers. Leaf photosynthesis of IR72 favoured higher growth temperatures more than M103. Rubisco total activity and protein content were negatively affected in both genotypes by high temperatures and elevated CO_2. However, at moderate to high growth temperatures, IR72 leaves averaged 71 and 39% more *rbcS* transcripts than M103 under ambient and elevated CO_2, respectively, and likewise had greater Rubisco activity and protein content. Expression of *psbA* (D1 protein of PSII) in IR72 leaves increased with temperature, whereas it remained constant for M103, except for a 20% decline at 40/30 °C under elevated CO_2. Even at the highest growth temperatures, PSII photochemical efficiency was not impaired in either genotype grown under either ambient or elevated CO_2. Genotypic differences exist in rice for carboxylation responses to elevated CO_2 and high temperatures, which may be useful in developing genotypes suited to cope with global climate changes.

Key-words: *Oryza sativa*; carbon assimilation; carbon dioxide; gene expression; heat-stress; photo-inhibition; ribulose 1, 5-bisphosphate carboxylase/oxygenase.

INTRODUCTION

The direct benefits of elevated atmospheric [CO_2] to plant growth and development, particularly for C_3 species, are well documented (Drake, Gonzàlez-Meler & Long 1997; Morison & Lawlor 1999). Largely, these benefits result from enhanced photosynthesis concomitant with reduced stomatal conductance (Cure & Acock 1986; Bowes 1993; Drake *et al*. 1997).

Atmospheric CO_2 and other 'greenhouse' gases (e.g. methane and nitrous oxide) are increasing, and as a consequence, the earth's mean annual surface temperature is also rising (Houghton *et al*. 2001). The earth's surface temperature has been estimated to have increased 0.6 °C just in the past three decades (Stott *et al*. 2000), and is predicted to increase as much as 1.4–5.8 °C within this century (Schneider 2001). High temperatures can inhibit photosynthetic carbon assimilation (Berry & Björkman 1980), and under such conditions some of the benefits of elevated atmospheric [CO_2] to plants may not be fully realized (Morison & Lawlor 1999).

Rice (*Oryza sativa* L.) is one of the most important world food crops (Matthews, Kropff & Bachelet 1995). In many crop-producing areas of the world, seasonal growth temperatures may often exceed the optimum for growth and development of plants. This is especially true for some of the major rice-producing areas in the tropics and subtropics. Rice, which is grown in most parts of the world, has become adapted to a variety of climates (Yoshida 1981). Few studies, however, have focused on intra-species differences of rice, or other agronomic crops, to interactive effects of long-term exposure to elevated [CO_2] and high temperatures. Ziska, Manalo & Ordonez (1996) grew 17 cultivars of rice including both indica and japonica types under 373 and 664 μmol CO_2 mol^{-1} and two temperature regimes representing an optimal (29/21 °C day/night) and high temperature (37/29 °C) scenario. They found considerable genotypic variation in plant growth and yield under the high temperature regime. Physiological mechanisms contributing to such differences were not measured in that study, although carbon assimilation and source/sink capacity were suggested as factors.

Based on present knowledge of Rubisco kinetics, it has been theorized that increased temperature under elevated [CO_2] should lead to greater photosynthetic productivity of C_3 plants (Long 1991). Rubisco in rice leaves (IR72, indica-

Correspondence: Russ W. Gesch. Fax: +1 320 589 3787; e-mail: gesch@morris.ars.usda.gov

type) has been found to be negatively affected by both elevated [CO_2] and temperature (Vu *et al*. 1997). Carboxylation capacity in rice is known to acclimate to [CO_2] (Rowland-Bamford *et al*. 1991), which may be primarily due to modulated expression of the small subunit gene (*rbcS*) of Rubisco (Gesch *et al*. 1998). However, the expression of *rbcS* in relation to high growth temperatures in concert with elevated [CO_2] is not well understood. For instance, Vu *et al*. (2001) grew soybean (*Glycine max* L.) under elevated [CO_2] at maximum daytime temperatures of 28, 32, 36, 40, 44, and 48 °C and found that leaf *rbcS* transcript abundance steadily declined between 28 and 44 °C, although Rubisco protein only began to decrease above 40 °C.

Acclimation to elevated [CO_2] resulting in decreased carboxylation capacity has been linked to reduced photochemical efficiency of PSII (Hymus *et al*. 2001) and furthermore, high temperatures have been shown to exacerbate this type of response (Roden & Ball 1996). However, in this regard, results are variable, indicating inter- and intraspecies differences (Roden & Ball 1996; Huxman *et al*. 1998).

Genetic modifications to important agronomic species such as rice will be necessary to cope with globally changing climates, especially in terms of heat-stress. In the following study we grew an indica-type rice (adapted to growth in tropical climates) and a japonica-type rice (adapted to temperate climates) cultivar side-by-side under ambient and elevated [CO_2] and at three temperature regimes to test the hypothesis that genotypic differences in photosynthetic response to elevated [CO_2] and temperature are related to protein and gene expression of Rubisco. Furthermore, PSII photochemistry and gene expression of the D1 protein of PSII (*psbA*) were examined to test the hypothesis that PSII photochemical efficiency is negatively affected by high growth temperatures under elevated [CO_2].

MATERIALS AND METHODS

Plant material and growth conditions

Rice (cvs. IR72 and M103) was grown in six outdoor environment-controlled chambers, known as Soil-Plant-Atmosphere-Research (SPAR) chambers, located in Gainesville, Florida (29°40′ N). These closed circulation chambers were constructed of an aluminium frame covered with transparent polyethylene telephtalate 'Sixlight' (Taiyo Kogyo Co., Tokyo, Japan) providing nearly natural solar radiation (Pickering *et al*. 1994). Above-ground chamber dimensions were 2.0 m × 1.0 m cross-section by 1.5 m height. A hinged extended volume of 0.5 m × 2.0 m cross-section by 1.2 m high was attached to each chamber. This section had a trap door with a drawstring body seal located on the bottom. This allowed a person equipped with a face mask ventilated to the outside of the chamber to measure leaf photosynthesis, while preventing CO_2 contamination. The chamber tops were attached to aluminium vats, 2.0 m × 1.0 m cross-section and 0.6 m deep, providing a water-tight, flooded, rooting environment for rice culture. The chamber vats were filled with a Kendrick fine sand topsoil to a depth of 0.5 m.

Seeds of IR72 (tropical indica-type) and M103 (temperate japonica-type) were directly sown by hand in five 1 m rows, spaced 18 cm apart, running north–south. A middle border row was sown with both cultivars. IR72 and M103 were sown on 23 and 25 August 1998, respectively, and grown under natural day-length. The east half of each chamber contained IR72 and the west half, M103. After 2 weeks, the plants were thinned to give a population of 275 plants m^{-2} for each cultivar. Based on soil analysis, phosphorous and potassium were incorporated into the top 0.15 m of soil at a rate of 8.4 and 13.5 g m^{-2}, respectively, prior to sowing. One week after sowing nitrogen as urea was applied at 12.6 g m^{-2}, followed by two split applications of 6.3 g m^{-2} at one-month intervals.

Three chambers of rice were grown under daytime atmospheric [CO_2] of 350 μmol mol^{-1} (ambient CO_2) and three others were grown at 700 μmol mol^{-1} (elevated CO_2). For each atmospheric CO_2 treatment, three diurnal growth temperature regimes were applied, consisting of day/night, maximum/minimum dry bulb temperatures of 28/18, 34/24 and 40/30 °C with corresponding dew point temperatures of 18/14, 24/20 and 30/26 °C, respectively. This gave maximum/minimum air vapour pressure deficits for the three temperature treatments of 1.7/0.5, 2.3/0.6, and 3.1/0.9 kPa. The diurnal temperature cycle was sinusoidal from 0700 to 1800 h eastern standard time (EST), with the minimum occurring at 0700 h, reaching the maximum at 1500 h, and then declining until 1800 h. Beginning at 1800 h EST, the temperature declined smoothly with an exponential decay function for the night until reaching the minimum at 0700 h the next day. Graphical representation of this type of diurnal temperature pattern used is shown by Vu *et al*. (2001). Parton & Logan (1981) found this diurnal temperature cycle to be a good representation of natural diurnal temperature patterns. Both dry bulb and dew point temperatures were controlled to within ±0.2 °C of their continuously cycling setpoints and daytime CO_2 concentrations were controlled to within ±5.0 μmol mol^{-1} of their setpoint.

Each chamber [CO_2] was monitored with a dedicated IR gas analyser (Ultramat 21P; Siemens, Alpharetta, GA, USA). Gas analysers were calibrated and checked for linearity before and after the experiment with a range of standard [CO_2] in nitrogen. Calibration was checked daily with a span gas. Microclimate data were collected every 2 s, and averages over 300 s were computed and recorded. All measurements of sensors and control activators were managed through a supervised control and data acquisition system (Pickering *et al*. 1994). Each chamber was controlled by an individual CR-10T controller/data acquisition device (Campbell Scientific Inc., Logan, UT, USA).

Photosynthesis and chlorophyll *a* fluorescence measurements

Prior to experimental sampling, 80 plants of each cultivar in each chamber were randomly chosen and tagged for use

in photosynthesis and chlorophyll fluorescence measurements and biochemical analyses. Photosynthesis was measured on the attached uppermost fully expanded leaf at the treatment growth [CO_2] and maximum or near maximum growth temperature with a LI-6200 Portable Photosynthesis System (LI-COR Inc., Lincoln, NE, USA) equipped with a 0.25 L cuvette. While making photosynthesis measurements, the LI-6200 leaf chamber temperatures varied as much as 0.8 °C below to as high as 2.5 °C above the maximum growth temperatures. Chlorophyll *a* fluorescence was also measured on the uppermost fully expanded leaf with a Walz PAM-2000 Portable Fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Chlorophyll fluorescence and photosynthesis measurements were taken between 1030 and 1300 h EST when solar photosynthetic photon flux density (PPFD) was saturating at ≥1200 μmol m^{-2} s^{-1}. Quantum yield of PSII (ϕ_{PSII}) was calculated from *in situ* measurements of F_m' (maximum) and F_s (steady-state) fluorescence according to Genty, Briantais & Baker (1989). The value of J_{PSII} (linear electron transport rate through PSII) was estimated from ϕ_{PSII} and assuming a leaf PPFD absorptance of 84% with 50% distributed to PSII. The maximum quantum yield of PSII (F_v/F_m) was calculated from measurements of F_o (minimum) and F_m (maximum) after dark-adapting the leaves for 15 min (Genty *et al*. 1989). For each treatment and cultivar, a minimum of three and four separate leaves were used for measurements of photosynthesis and fluorescence, respectively.

Leaf sampling

At 58 d after planting (DAP) for IR72 and 56 DAP for M103, the uppermost fully expanded leaves from 10 to 15 plants were detached and immediately immersed in liquid nitrogen. Leaf sampling was done between 1130 and 1230 h EST. Sampled leaves were pooled by treatment, ground to a fine powder in liquid nitrogen and stored in liquid nitrogen until analysed. A subset of three leaves per treatment was taken for determination of fresh weight to leaf area ratio. Leaf area was measured with a LI-3100 leaf area meter (LI-COR). Temperature, but not [CO_2], affected leaf number. The leaf number on the main culm sampled for M103 and IR72 plants, respectively, was 7 and 8 for the 28/18 °C temperature treatment, and 8 and 9 for both the 34/24 and 40/30 °C treatments. At 34/24 and 40/30 °C, M103 plants were undergoing panicle emergence, whereas those of IR72 were at the panicle initiation stage of development. At 28/18 °C, M103 plants were at panicle initiation, while those of IR72 were in the late vegetative stage of development.

RNA isolation and Northern analysis

Total RNA was isolated from 300 mg of liquid-nitrogen-frozen leaf tissue using the phenol/chloroform extraction method of De Vries, Hodge & Bisseling (1988). Total RNA was quantified by UV spectrophotometry and 5 μg samples were separated electrophoretically on 1% agarose gels and transferred by Northern blotting to 'Hybond-N' nylon membranes (Amersham Biosciences, Piscataway, NJ, USA) using the procedures of Sambrook, Fritsch & Maniatis (1989).

Detection of mRNA for *rbcS* (Rubisco small subunit gene) and *psbA* (D1 protein of PSII gene) was performed by hybridization of ^{32}P-labelled DNA probes. Probes were made from fragments of cDNA of rice *rbcS* (Xie & Wu 1988) and barley (*Hordeum vulgare* L.) *psbA* (Rapp, Baumgartner & Mullet 1992) labelled with α-^{32}P dCTP using the Prime-a Gene® labelling system (Promega, Madison, WI, USA). Hybridizations were performed at 65 °C in 250 mM sodium phosphate buffer containing 7% sodium dodecyl sulphate and 100 μg mL^{-1} denatured salmon sperm DNA. After washing, membranes were scanned and quantified by phosphorimaging (Molecular Dynamics, Sunnyvale, CA, USA). Labelled 25S radish ribosomal RNA gene fragments (Delseny, Cooke & Penon 1983) were used as an internal standard and to verify equal loading. Northern analysis was performed three separate times.

Rubisco activity and protein content assays

Rubisco total activity was determined by the method of Gesch *et al*. (1998). Approximately 150 mg of liquid-nitrogen-frozen leaf tissue was extracted at 4 °C in 3 mL of medium containing 50 mM CO_2-free Tricine-NaOH pH 8.0, 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 10 mM isoascorbate, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 2% (w/v) polyvinyl-pyrrolidone (PVP)-40. The homogenate was microcentrifuged for 45 s at 4 °C and an aliquot of the supernatant was immediately assayed for Rubisco activity at 30 °C. The reaction mixture contained 50 mM CO_2-free Tricine-NaOH pH 8.0, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM EDTA, 0.5 mM ribulose 1,5-biphosphate (RuBP), and 10 mM $NaH^{14}CO_3$ (2 Gbq $mmol^{-1}$). The assays were dried at 60 °C and the acid-stable ^{14}C radioactivity determined by scintillation spectrometry. A 0.1 mL aliquot of the initial leaf extract prior to centrifugation was used to determined chlorophyll content in 80% (v/v) acetone using the extinction coefficients of Arnon (1949).

Rubisco protein content was determined by a radioimmuno-precipitation method described by Vu *et al*. (1997). To a 0.2 mL aliquot of the leaf extract obtained from the Rubisco activity assays, $NaHCO_3$ was added to 10 mM and the mixture allowed to incubate on ice for 20 min to activate Rubisco. A 0.025 mL aliquot of this mixture was added to 0.05 mL of buffer (100 mM bicine pH 7.8, 20 mM $MgCl_2$, 1 mM EDTA) containing 4 nmol [2-^{14}C] carboxyarabinitol bisphosphate (CABP) and 0.05 mL of antiserum to purified tobacco Rubisco raised from rabbits. After incubation at 37 °C for 2 h, the precipitate was collected and the amount of ^{14}C was determined by liquid scintillation spectrometry. Assays were performed in triplicate on two extractions per treatment.

Statistical analysis

The experimental design was a random incomplete block without replication of CO_2 by temperature combinations.

A single-factor nested analysis of variance (ANOVA) was used to determine [CO_2] differences over all temperatures and cultivars, with treatment chambers nested within [CO_2] and measurements of the dependent variable nested within chambers. A single-factor ANOVA was used to determine differences between cultivars at each temperature. In both of these cases, the PROC MIXED procedure (SAS Institute, Cary, NC, USA) was used for the analysis. A general linear model (GLM) *F*-test for full and reduced models was used to test for cultivar differences over all temperature and CO_2 levels. The PROC REG procedure of SAS was used for this analysis and to determine whether there were significant CO_2–temperature interactions for each cultivar. The general form of the equations expressed the dependent variable as a function of CO_2, temperature, CO_2–temperature interaction.

RESULTS

During the life cycle of plants, leaf photosynthesis for IR72 generally was greatest under the 34/24 °C growth temperature regime, whereas that of the temperate cultivar M103 favoured the 28/18 °C treatment (Fig. 1a & b). For both cultivars, leaf photosynthesis tended to change little during development under most growth [CO_2] and temperature regimes, indicating acclimation of plants to their respective treatments (Fig. 1a & b). An exception, however, was M103 grown under elevated CO_2 at 40/30 °C, which showed a consistent and dramatic decline in photosynthesis throughout development (Fig. 1b).

Temperature, but not [CO_2], significantly affected the development of both cultivars (Snyder 2000). The time from sowing to 50% anthesis at the 28/18, 34/24, and 40/30 °C treatments were 110, 85, and 91 d for IR72 and 81, 58, and 63 d for M103 (data not shown). Both cultivars in the present study showed the same pattern of developmental response to temperature, with IR72 taking an average of 28 d longer to reach 50% anthesis (Snyder 2000). The developmental response of rice between 28/18 and 34/24 °C observed in our study was similar to that reported by Baker, Allen & Boote (1992) for the cultivar IR30 grown at 28/21 and 34/27 °C day/night temperatures, sown on 10 October.

Leaf photosynthetic trends at 58 DAP for IR72 and 56 DAP for M103 with respect to temperature were similar under ambient and elevated growth [CO_2] (Fig. 2). However, changes in photosynthesis with temperature were much greater under elevated. Under both CO_2 regimes at 28 °C growth temperature, photosynthesis in M103 leaves

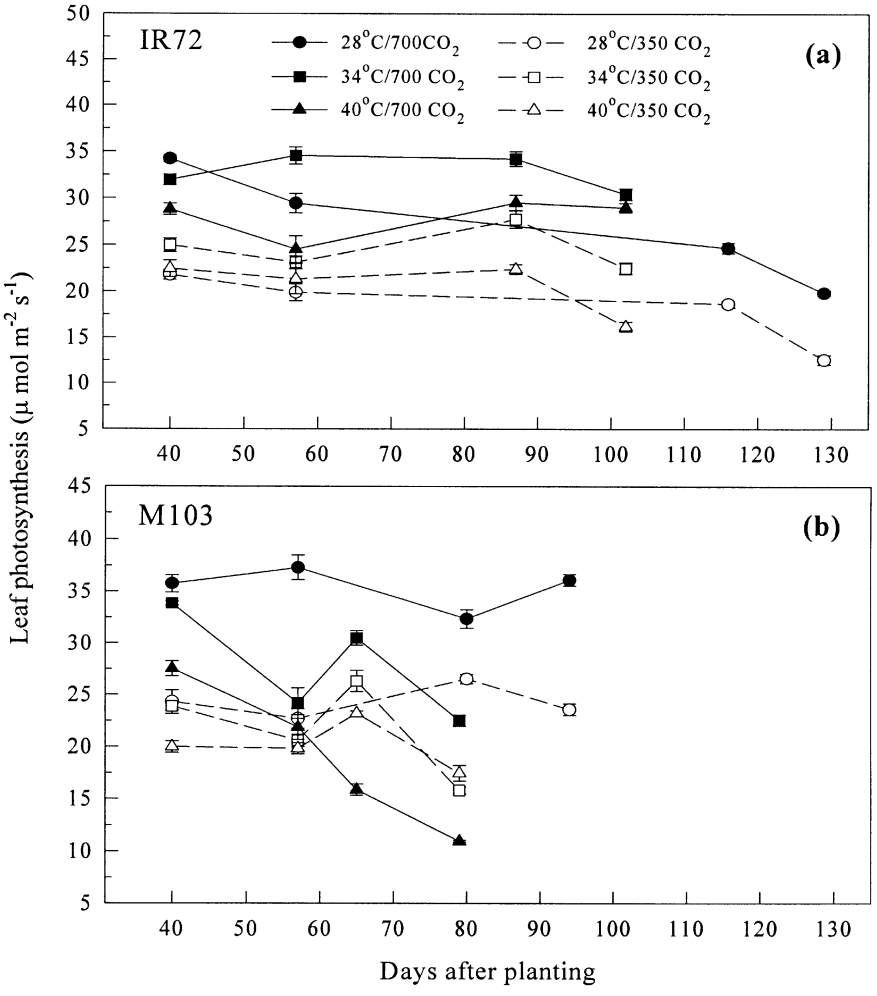

Figure 1. Leaf photosynthesis for IR72 (a) and M103 (b) during growth of plants under ambient (open symbols) and elevated (closed symbols) CO_2. Measurements were made at growth [CO_2] and at or near maximum growth temperature, which are given beside each symbol. Values are the mean ±SE of three separate leaves per treatment. The last two data points for each cultivar by treatment were measured on flag leaves at approximately 50% anthesis and 2 weeks later.

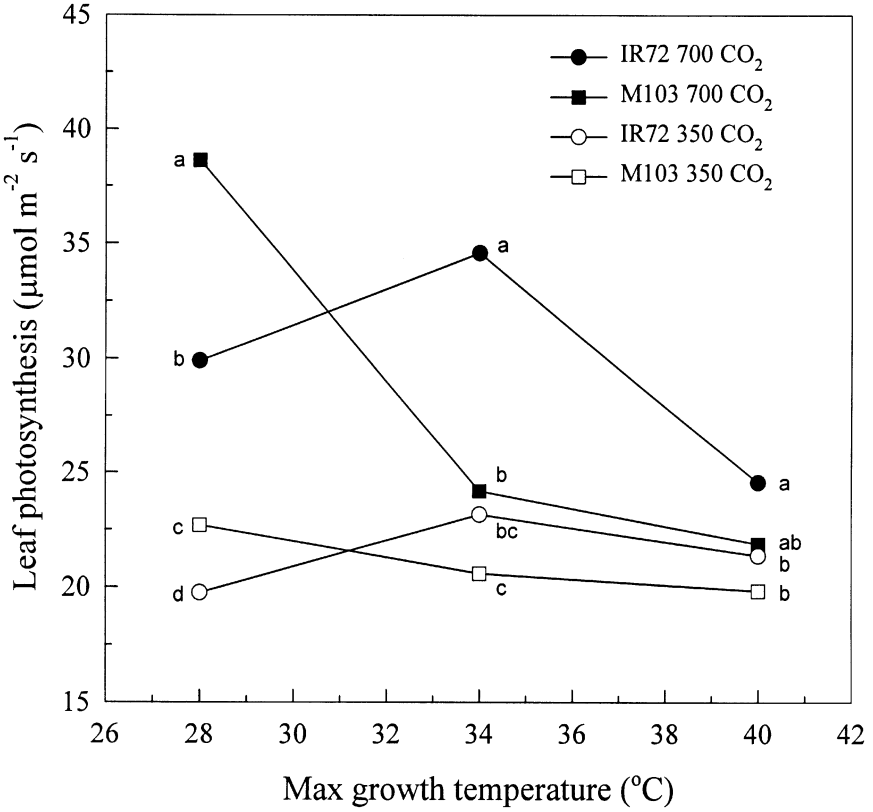

Figure 2. Leaf photosynthesis as a function of maximum growth temperature at 58 and 56 d after planting (DAP) for IR72 and M103, respectively, grown under ambient and elevated CO_2. Measurements were made at growth [CO_2] and at or near maximum growth temperature. Values are the mean of three separate leaves per treatment. Data points within a temperature followed by the same letter are not statistically different at the $P \leq 0.05$ level.

was greater than in IR72 ($P < 0.05$). At 34 °C and elevated CO_2, photosynthesis was greater for IR72 ($P < 0.05$), but under ambient CO_2 was only marginally higher than M103 (Fig. 2). Under both ambient and elevated CO_2, there was no significant difference between the cultivars at 40 °C (Fig. 2). With respect to atmospheric growth [CO_2], photosynthesis for both IR72 and M103 was significantly greater ($P < 0.05$) under elevated than ambient CO_2 at 28 and 34 °C growth temperature, but at 40 °C, was only statistically different for IR72 (Fig. 2).

To compare *rbcS* and *psbA* transcript abundance between cultivars as a function of increasing temperature and [CO_2], the comparisons were made relative to transcript abundance measured for IR72 leaves grown at ambient CO_2 and 28 °C (Table 1). At ambient CO_2 and 28 °C, *rbcS* transcript amounts in mature leaves of IR72 and M103 were nearly identical (Table 1). In contrast to photosynthesis, changes in *rbcS* expression with temperature were more pronounced under ambient than elevated CO_2. As growth temperature increased to 34 °C, *rbcS* expression rose 44% in IR72 leaves grown under ambient CO_2, while it declined 31% in those of M103, and essentially remained at these

Table 1. Percentage *rbcS* and *psbA* leaf mRNA abundance in leaves of M103 and IR72 in response to growth temperature and [CO_2]

		Growth [CO_2]			
	Max. growth temperature	350 (μmol mol^{-1})		700 (μmol mol^{-1})	
mRNA	(°C)	M103	IR72	M103	IR72
% *rbcS*	28	101 ± 8a	100a	77 ± 4b	106 ± 10a
	34	69 ± 1c	144 ± 8a	74 ± 11c	101 ± 2b
	40	70 ± 10c	137 ± 3a	62 ± 12c	112 ± 8b
% *psbA*	28	102 ± 4a	100a	97 ± 4a	80 ± 2b
	34	92 ± 5a	102 ± 2a	102 ± 3a	99 ± 4a
	40	95 ± 4b	114 ± 6a	84 ± 5c	112 ± 3a

Leaves were sampled at 56 and 58 DAP for M103 and IR72, respectively. Values are calculated as the mean ± SE percentage relative to leaves of IR72 grown under ambient CO_2 at 28/18 °C day/night temperature. Northern analysis was performed three separate times. Values within a temperature treatment followed by the same letter are not statistically different at the $P \leq 0.05$ level.

Table 2. Rubisco total activity and protein content in leaves of M103 and IR72 in response to growth temperature and [CO_2]

Rubisco	Max. growth temperature (°C)	Growth [CO_2] 350 (μmol mol^{-1}) M103	350 (μmol mol^{-1}) IR72	700 (μmol mol^{-1}) M103	700 (μmol mol^{-1}) IR72
Total activity (μmol m^{-2} s^{-1})	28	79.3 ± 0.9a	73.8 ± 0.8b	65.8 ± 0.7c	63.6 ± 1.0d
	34	38.5 ± 0.3c	53.7 ± 0.4a	37.6 ± 0.9c	44.3 ± 1.3b
	40	43.9 ± 0.4b	55.1 ± 0.6a	20.0 ± 0.3c	45.4 ± 0.7b
Protein (g m^{-2})	28	2.44 ± 0.11a	2.16 ± 0.03b	2.06 ± 0.05bc	1.95 ± 0.03c
	34	1.15 ± 0.03c	1.65 ± 0.05a	1.15 ± 0.04c	1.48 ± 0.06b
	40	1.29 ± 0.04c	1.73 ± 0.02a	0.64 ± 0.01d	1.47 ± 0.04b

Leaves were sampled at 56 and 58 DAP for M103 and IR72, respectively. Values are the mean ± SE of two separate extractions assayed three times each. Values within a temperature treatment followed by the same letter are not statistically different at the $P \leq 0.05$ level.

values at 40 °C (Table 1). Under ambient CO_2, *rbcS* transcript amounts in IR72 leaves at 34 and 40 °C were significantly greater ($P < 0.05$) than those in M103 leaves (Table 1). Under elevated CO_2, temperature had little impact on *rbcS* expression for either rice cultivar. However, IR72 maintained significantly greater ($P < 0.05$) *rbcS* transcript abundance than M103 across all temperature treatments (Table 1).

Expression of the chloroplast encoded *psbA* gene was similar for both cultivars and CO_2 levels at 28 °C with the exception that the transcript amount in IR72 leaves grown under elevated CO_2 was approximately 20% less (Table 1). Under both growth CO_2 regimes, *psbA* expression tended to increase in IR72 leaves as growth temperature increased. For IR72 leaves developed under elevated CO_2 the increase from 28 to 40 °C was 32% (Table 1). Leaves of M103 grown under ambient CO_2 showed no significant change in *psbA* expression with increasing temperature, whereas those at elevated CO_2 showed about a 20% decline as maximum growth temperature rose from 34 to 40 °C (Table 1). Under elevated CO_2 and 28 °C, M103 leaves expressed a greater ($P < 0.05$) abundance of *psbA* transcripts than those of IR72. Though *psbA* expression was not significantly different between cultivars at 34 °C, at 40 °C IR72 leaves showed significantly greater ($P < 0.05$) expression than M103 under both ambient and elevated CO_2 (Table 1).

High temperatures negatively affected Rubisco total activity and enzyme protein content in leaves of both cultivars, but the response tended to be greater in M103 (Table 2). At 28 °C, M103 leaves exhibited greater ($P < 0.05$) Rubisco activity than IR72 (Table 2). But at higher temperatures, IR72 maintained greater ($P < 0.05$) Rubisco activity than M103 under both ambient and elevated CO_2 (Table 2). IR72 leaves grown at 40/30 °C under elevated CO_2 had as much as 2.3-fold greater total Rubisco activity than those of M103 (Table 2). Rubisco protein content mirrored total activity (Table 2). For both cultivars across temperature treatments, Rubisco total activity and protein content were generally greater under ambient than elevated CO_2, except that there was no difference between CO_2 treatments for M103 at 34 °C (Table 2). Across temperatures, Rubisco protein in leaves of ambient CO_2-grown IR72 plants averaged 12% greater than those grown under elevated CO_2 (Table 2). For M103 at 40 °C, ambient CO_2-grown plants had 50% more Rubisco protein in their leaves than those under elevated CO_2, whereas at 28 °C it was only 15% greater (Table 2).

Table 3 summarizes results for testing the overall effects of rice cultivar, [CO_2], temperature, and [CO_2] by temperature interaction on leaf photosynthesis, Rubisco, and *psbA* expression. Across CO_2 levels and all temperatures, the two cultivars significantly differed for all the response variables shown. The [CO_2] by temperature interaction was significant for photosynthesis and *psbA* expression for both cul-

Table 3. Results of statistical analysis to test whether the main effects Cultivar, atmospheric [CO_2] (CO_2), and the interaction of CO_2 with temperature ($CO_2 \times$ Temp) significantly affected photosynthesis, Rubisco total activity and protein, and *rbcS* and *psbA* transcript levels. For Cultivar and CO_2 the analysis was done over all [CO_2], cultivars, and temperatures. For Temp and $CO_2 \times$ Temp, analysis was for each cultivar over all [CO_2] and temperatures. See Material and Methods for statistical procedures used

Source	Photosynthesis	Rubisco total activity	Rubisco protein content	*rbcS*	*psbA*
Cultivar	**	**	**	**	**
CO_2	*†	NS	NS	NS	NS
Temp	NS IR72, NS M103	NS IR72, NS M103	NS IR72, *M103	NS IR72, NS M103	NS IR72, NS M103
$CO_2 \times$ Temp	**IR72, **M103	NS IR72, **M103	NS IR72, NS M103	**IR72, NS M103	**IR72, *M103

*, **Denote significant effect at $P \leq 0.05$, and 0.01 levels, respectively. *† Denotes significant effect at $P \leq 0.06$ level. NS denotes non-significance at these levels.

Table 4. Total chlorophyll content in leaves of M103 and IR72 in response to growth temperature and [CO_2]

Max. growth temperature (°C)	Growth [CO_2] 350 (μmol mol^{-1}) M103	350 (μmol mol^{-1}) IR72	700 (μmol mol^{-1}) M103	700 (μmol mol^{-1}) IR72
28	622 ± 11a	569 ± 10b	570 ± 13b	514 ± 9c
34	438 ± 3b	435 ± 4b	501 ± 2a	442 ± 16b
40	451 ± 8a	450 ± 3a	367 ± 2b	462 ± 1a

Leaves were sampled at 56 and 58 DAP for M103 and IR72, respectively. Values are given as [Chl] mg m^{-2} and are the mean ± SE of two separate extractions assayed twice each. Values within a temperature treatment followed by the same letter are not significantly different at the $P \leq 0.05$ level.

tivars, but varied with respect to Rubisco total activity and *rbcS* expression, and was not significant for Rubisco protein content (Table 3).

Chlorophyll (Chl) content responded to temperature similarly to that of Rubisco. For both cultivars and CO_2 treatments there was a substantial decline in Chl content with an increase of maximum growth temperature to 34 °C, but there was no further decrease at 40 °C, except for M103 grown under elevated CO_2 (Table 4). At 28 °C, Chl content was significantly greater ($P < 0.05$) in M103 leaves than IR72 under both CO_2 treatments. At 34 and 40 °C, there was no difference in Chl content between the cultivars grown at ambient CO_2, but differences were apparent at elevated CO_2. Thus, Chl in M103 leaves was greater ($P < 0.05$) than in IR72 at 34 °C, but significantly less ($P < 0.05$) at 40 °C (Table 4). Under elevated CO_2 at 40 °C, M103 leaves contained 36% less Chl than those from plants grown under ambient CO_2 (Table 4).

Photochemical efficiency of PSII, as assessed by F_v/F_m, was affected very little in leaves of either rice cultivar by high temperature and elevated CO_2 (Table 5). For M103, electron transport rate through PSII (J_{PSII}) tended to be greater for leaves grown under ambient CO_2, and J_{PSII} in leaves of IR72 developed under elevated CO_2 tended to increase between 28 and 40 °C (Table 5). However, neither relationship was statistically significant ($P < 0.05$). For quantum yield of PSII, again, no significant differences between cultivars, or interactions with CO_2 and temperature were apparent in leaves of rice at 48 DAP (Table 5).

DISCUSSION

Under ambient atmospheric CO_2, increasing growth temperatures from 28/18 to 40/30 °C had only a small effect on leaf photosynthesis, although IR72 showed a preference for higher growth temperatures than M103 (Fig. 2). However, when rice was grown under elevated CO_2, the effect of temperature was much greater. Identifying physiological factors responsible for this intraspecific difference in photosynthesis will aid in developing strategies for improving cultivar response to persistent increases in atmospheric [CO_2] and aerial temperatures.

It was hypothesized that photosynthetic differences to high growth temperatures and [CO_2] in these rice cultivars may be related to the expression of Rubisco. Generally, our results support this hypothesis. Although growth temperatures above 28/18 °C negatively affected Rubisco total activity and protein content in the leaves of both cultivars, IR72 consistently maintained significantly greater levels than M103. This was due, at least in part, to higher maintenance of Rubisco synthesis above 28/18 °C as indicated by greater *rbcS* expression in IR72 leaves. Also, rice in this study showed photosynthetic acclimation to elevated CO_2 (i.e. down-regulation of Rubisco) as previously reported (Rowland-Bamford *et al*. 1991; Gesch *et al*. 1998), although the M103 response was more variable, with little difference between CO_2 treatments at the 34/24 °C temperature regime.

There was not always a good association between *rbcS* mRNA levels and Rubisco protein content. Under ambient CO_2, at 34/24 °C transcript abundance for IR72 leaves was about 40% greater than those of plants at 28/18 °C even

Table 5. Effects of 28/18 and 40/30 °C growth temperature treatments under ambient and elevated CO_2 on leaf maximum PSII quantum yield (F_v/F_m), PSII electron transport (J_{PSII}), and light adapted quantum yield (ϕ_{PSII}) at 49 and 47 DAP for IR72 and M103, respectively

Attribute	Max. growth temperature (°C)	Growth [CO_2] 350 (μmol mol^{-1}) M103	350 (μmol mol^{-1}) IR72	700 (μmol mol^{-1}) M103	700 (μmol mol^{-1}) IR72
F_v/F_m	28	0.74 ± 0.02a	0.70 ± 0.01a	0.75 ± 0.01a	0.70 ± 0.02a
	40	0.71 ± 0.04a	0.67 ± 0.02a	0.70 ± 0.01a	0.72 ± 0.02a
J_{PSII} (μmol m^{-2} s^{-1})	28	151 ± 9a	133 ± 7ab	129 ± 15ab	115 ± 6b
	40	135 ± 16a	123 ± 12a	120 ± 9a	138 ± 6a
ϕ_{PSII}	28	0.31 ± 0.02a	0.31 ± 0.03a	0.32 ± 0.05a	0.28 ± 0.02a
	40	0.31 ± 0.05a	0.29 ± 0.04a	0.31 ± 0.02a	0.36 ± 0.03a

Measurements were made at growth [CO_2] and at or near maximum growth temperature. Values are the mean ± SE of four separate leaves per treatment. Values within temperature treatments followed by the same letter are not statistically significant at the $P \leq 0.05$ level.

though Rubisco protein was lower. Based on our findings, it is not clearly evident why this occurred. However, effects of temperature on source/sink balance of plants may have been a factor. Sink (i.e. growing tissues and organs) activity has been implicated in the regulation of photosynthetic gene expression, including *rbcS* (Koch 1996). Sink-limited conditions are often associated with repression of *rbcS* expression and vice-versa under high sink demand for photosynthate (Koch 1996; Moore *et al*. 1999). In the present study, IR72 under ambient CO_2, grew and developed faster at 34/24 °C than at 28/18 °C and therefore likely had greater sink strength at the time leaves were sampled. This in turn could have triggered greater *rbcS* expression. A similar response was not observed in M103 plants grown under ambient CO_2, however, their growth was adversely affected by temperatures of 34/24 °C and higher (Snyder 2000).

A lack of correlation between *rbcS* transcript abundance and Rubisco content has been observed by others for various plant species (Moore *et al*. 1998; Vu *et al*. 2001), and likely reflects the complex nature of the molecular regulation of Rubisco. Several factors effect the regulation of Rubisco synthesis and amount of enzyme protein including transcription, post-transcription message stability, translation and protein turn-over, which all may potentially be affected by growth [CO_2] (Webber, Nie & Long 1994) and leaf developmental stage (Deng & Gruissem 1987; Gesch *et al*. 1998; Suzuki, Makino & Mae 2001). Although the leaf number sampled from the main culm differed by one between cultivars and between the low and higher temperature regimes, care was taken to sample leaves at the same stage of development across treatments. Suzuki *et al*. (2001) examined Rubisco synthesis and degradation, and *rbcS* and *rbcL* transcript levels in developing eighth-leaves of rice. They found that *rbcS* mRNA and Rubisco synthesis peaked about mid-way through leaf expansion with the greatest amount of protein occurring just prior to full expansion. They also showed that *rbcS* mRNA began decreasing rapidly before full expansion and that Rubisco content in mature leaves (i.e. after full expansion) was more dependent on degradation than synthesis. Leaves in our study were sampled at full expansion, when Rubisco content was probably near its highest level but not *rbcS* mRNA. Nevertheless, greater amounts of Rubisco protein and *rbcS* mRNA indicate greater synthesis in IR72 leaves than M103 under high growth temperatures and elevated CO_2.

At 40/30 °C, M103 leaf Rubisco total activity and content were more sensitive under elevated than ambient CO_2 (Table 2). Reasons for this are not fully known, but again, may reflect changes to plant source/sink balance imparted by temperature and [CO_2]. At 40/30 °C, biomass production and growth rate of M103 plants under elevated CO_2 were lower than that for those grown under ambient CO_2 (Snyder 2000), and thus were presumably more sink limited. If this were the case, reduced sink capacity in these plants could have led to enhanced feedback inhibition of photosynthesis and subsequent down-regulation of Rubisco activity and content (Bowes 1993; Webber *et al*. 1994).

Coarse control of Rubisco (i.e. total activity and content) was not the only factor limiting temperature and CO_2-induced changes in rice leaf photosynthesis. In some instances changes in leaf photosynthesis did not correspond to changes in Rubisco total activity. Under ambient CO_2, photosynthesis of both cultivars changed little with increasing growth temperature, despite large reductions in Rubisco activity and content. According to the model of Farquhar, von Caemmerer & Berry (1980), leaf photosynthesis of C_3 plants is primarily limited by Rubisco at or below normal ambient atmospheric [CO_2], and by RuBP regeneration at high[CO_2]. In our study, leaf Rubisco content under ambient CO_2, despite being reduced by high temperatures, may have been sufficient to maintain *in situ* photosynthesis with only small changes. Murchie *et al*. (2002) measured light saturated rates of photosynthesis at 350 mol mol^{-1} in the flag leaves of several rice cultivars during flowering and grain filling. Over about a 20-d period they found that photosynthesis in all cultivars either did not change or decreased only slightly, even though some lost as much as 70% of their leaf Rubisco content during this time.

Under elevated CO_2, both IR72 and M103 showed greater sensitivity of leaf photosynthesis to temperature. Leaf stomatal conductance, which often decreases with elevated growth [CO_2] (Drake *et al*. 1997), but can vary with increasing temperature (Berry & Björkman 1980), did not appear to be a limiting factor here. The C_i/C_a ratio for both cultivars across treatments only ranged from 0.86 to 0.93 (data not shown), and except for a slight (5%) increase at the highest temperature treatment, there was no discernible trend with either [CO_2] or temperature.

Although not determined in this study, it is suggested here that effects of [CO_2] and temperature on Rubisco activation and RuBP regeneration might explain the degree of photosynthetic change reported in this study for plants grown under elevated CO_2. Increased leaf photosynthesis for IR72 between 28 and 34 °C under elevated CO_2 could have been caused by increased RuBP regeneration rate. Photosynthetic electron transport rate and triose-phosphate utilization are believed to be primary factors controlling RuBP regeneration (Farquhar *et al*. 1980; Sage 1990). Electron transport rate likely did not significantly affect RuBP regeneration since J_{PSII} changed only slightly in IR72 leaves between 28 and 40 °C (Table 5). However, the growth rate and development of IR72 under elevated CO_2 was greater at 34 than 28 °C (Snyder 2000). Therefore, it is highly probable that triose-phosphate utilization was greater at 34 °C, thus resulting in higher RuBP regeneration rate and photosynthesis. Within a maximum growth temperature range of 32–38 °C under 660 μmol mol^{-1} [CO_2], Vu *et al*. (1997) found that rice leaf photosynthesis increased from 32 to 35 °C followed by a decline at 38 °C. Yet within the temperature range of 32–38 °C, Rubisco content decreased 3.8% for each 1 °C rise.

High temperatures and elevated [CO_2] can reduce Rubisco activation (Kobza & Edwards 1987; Sage, Sharkey & Seemann 1990; Crafts-Brandner & Salvucci 2000).

Rubisco de-activation in rice leaves with increasing temperature has been shown to be greater under high than low atmospheric growth [CO_2] (Vu *et al*. 1997). In our study, under elevated CO_2, M103 leaf photosynthesis decreased sharply at lower growth temperatures (34/24 °C) than IR72 (40/30 °C). It is tempting to speculate here that this variation in sensitivity to elevated CO_2 and temperature between cultivars may in part be caused by differences in Rubisco de-activation. However, further research will be necessary to determine whether genotypic differences in rice exist for fine control of Rubisco activity under elevated growth CO_2 and temperature.

PSII photochemistry was hardly affected in rice leaves by the CO_2 and temperature treatments. Despite decreased leaf chlorophyll content for both rice cultivars with increased growth temperature, there was no evidence that either elevated CO_2 or high temperatures significantly impaired or enhanced PSII photochemical efficiency. High temperatures may reduce PSII photochemical efficiency in higher plants (Havaux 1992) and elevated [CO_2] may exacerbate such a response (Roden & Ball 1996). Conversely, evidence has also been presented that PSII photochemical efficiency is unaffected or even enhanced by elevated [CO_2] under high temperatures (Huxman *et al*. 1998). However, these variations are likely to be both species specific and dependant on experimental procedures.

Photo-inhibition of PSII, in conjunction with temperature extremes, can accelerate D1 protein damage, hence contributing to decreased electron transport efficiency (Demmig-Adams & Adams 1992). Given favourable temperatures and adequate light, the turn-over rate of the D1 protein of PSII is relatively rapid (Tyysjärvi, Mäenpää & Aro 1994), but environmental perturbations may inhibit the D1 repair mechanism (Demmig-Adams & Adams 1992). Evidence from the present study indicates that D1 repair was not a factor for either rice cultivar at high temperature or [CO_2]. Gene expression for D1 protein (i.e. *psbA* transcript abundance) increased with temperature in leaves of IR72 and at the highest temperature treatment was significantly greater than M103. There was no clear trend in *psbA* transcript abundance for M103 except for a 20% decrease at 40/30 °C under elevated CO_2. However, even at the highest growth temperatures photochemical efficiency and electron transport through PSII were only slightly affected. These results seem to indicate that both rice cultivars were able to acclimate photochemical processes to supra-optimal growth temperature and elevated CO_2.

If the earth's surface temperatures continue to increase with rising atmospheric CO_2 clearly there will be a need for improved heat tolerance of agronomic crops. For rice, genotypic differences exist for carboxylation responses to elevated CO_2 and high temperatures, and this may be useful in developing genotypes suited to cope with global climate changes. Further work is needed to identify underlying mechanisms regulating photosynthetic gene expression and Rubisco activity in plants acclimated to long-term exposures of high temperatures and elevated CO_2.

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REFERENCES

Arnon D.I. (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24,** 1–10.

Baker J.T., Allen L.H. Jr & Boote K.J. (1992) Response of rice to carbon dioxide and temperature. *Agricultural and Forest Meteorology* **60,** 153–166.

Berry J. & Björkman O. (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31,** 491–543.

Bowes G. (1993) Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* **44,** 309–332.

Crafts-Brandner S.J. & Salvucci M.E. (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *Proceedings of the National Academy of Sciences of the USA* **97,** 13430–13435.

Cure J.D. & Acock B. (1986) Crop responses to carbon dioxide doubling: a literature survey. *Agricultural and Forest Meteorology* **38,** 127–145.

De Vries S.D., Hodge H. & Bisseling T. (1988) Isolation of total and polysomal RNA from plant tissues. *Plant Molecular Biology Manual* **B6,** 1–13.

Delseny M., Cooke R. & Penon P. (1983) Sequence heterogeneity in radish nuclear ribosomal RNA genes. *Plant Science Letters* **30,** 107–119.

Demmig-Adams B. & Adams W.W. (1992) Photoprotection and other reponses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology* **43,** 599–626.

Deng X.-W. & Gruissem W. (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. *Cell* **49,** 379–387.

Drake B.G., Gonzàlez-Meler M.A. & Long S.P. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 609–639.

Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Genty B., Briantais J.-M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

Gesch R.W., Boote K.J., Vu J.C.V., Allen L.H. Jr & Bowes G. (1998) Changes in growth CO_2 result in rapid adjustments of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene expression in expanding and mature leaves of rice. *Plant Physiology* **118,** 521–529.

Havaux M. (1992) Stress tolerance of photosystem II in vivo: Antagonistic effects of water, heat, and photoinhibition stresses. *Plant Physiology* **100,** 424–432.

Houghton J.T., Ding Y., Griggs D.J., Noguer M., van der Linden P.J., Dai X., Maskell K. & Johnson C.A. (eds) (2001) *Climate Change 2001: The Scientific Basis. Report of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge University Press, Cambridge, UK.

Huxman T.E., Hamerlynck E.P., Loik M.E. & Smith S.D. (1998) Gas exchange and chlorophyll fluorescence responses of three southwestern Yucca species to elevated CO_2 and high temperature. *Plant, Cell and Environment* **21,** 1275–1283.

Hymus G.J., Dijkstra P., Baker N.R., Drake B.G. & Long S.P. (2001) Will rising CO_2 protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons. *Plant, Cell and Environment* **24,** 1361–1368.

Kobza J. & Edwards G.E. (1987) Influences of leaf temperature on photosynthetic carbon metabolism in wheat. *Plant Physiology* **83,** 69–74.

Koch K.E. (1996) Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47,** 509–540.

Long S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? *Plant, Cell and Environment* **14,** 729–739.

Matthews R.B., Kropff M.J. & Bachelet D. (1995) Introduction. In *Modeling the Impact of Climate Change on Rice Production in Asia* (eds R.B. Matthews, M.J. Kropff, D. Bachelet & H.H. Van Laar), pp. 3–7. CAB International, Wallingford, UK.

Moore B.D., Cheng S.-H., Rice J. & Seemann J.R. (1998) Sucrose cycling, Rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell and Environment* **21,** 905–915.

Moore B.D., Cheng S.-H., Sims D. & Seemann J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell and Environment* **22,** 567–582.

Morison J.I.L. & Lawlor D.W. (1999) Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant, Cell and Environment* **22,** 659–682.

Murchie E.H., Yang J., Hubbart S., Horton P. & Peng S. (2002) Are there associations between grain-filling rate and photosynthesis in flag leaves of field-grown rice? *Journal of Experimental Botany* **53,** 2217–2224.

Parton W.J. & Logan J.A. (1981) A model for diurnal variation in soil and air temperature. *Agricultural and Forest Meteorology* **23,** 205–216.

Pickering N.B., Allen L.H. Jr, Albrecht S.L., Jones P.J., Jones J.W. & Baker J.T. (1994) Environmental plant chambers: Control and measurement using CR-10T dataloggers. In *Computers in Agriculture. Proceedings of the 5th International Conference on Computers in Agriculture, Orlando, Florida* (eds D.G. Watson, F.S. Zazueta & T.V. Harrison), pp. 29–35. American Society of Agricultural Engineers, St Joseph, MI, USA.

Rapp J.C., Baumgartner B.J. & Mullet J. (1992) Quantitative analysis of transcription and RNA levels of 15 barley chloroplast genes. *Journal of Biology Chemistry* **267,** 21404–21411.

Roden J.S. & Ball M.C. (1996) The effect of elevated [CO_2] on growth and photosynthesis of two Eucalyptus species exposed to high temperatures and water deficits. *Plant Physiology* **111,** 909–919.

Rowland-Bamford A.J., Baker J.T., Allen L.H. Jr & Bowes G. (1991) Acclimation of rice to changing atmospheric carbon dioxide concentration. *Plant, Cell and Environment* **14,** 577–583.

Sage R.F. (1990) A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport, and triose phosphate use in response to light intensity and CO_2 in C_3 plants. *Plant Physiology* **94,** 1728–1734.

Sage R.F., Sharkey T.D. & Seemann J.R. (1990) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to light intensity and CO_2 in C_3 annuals *Chenopodium album* L. and *Phaseolus vulgaris* L. *Plant Physiology* **94,** 1735–1742.

Sambrook J., Fritsch E.F. & Maniatis T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, NY, USA.

Schneider S.H. (2001) What is 'Dangerous' climate change? *Nature* **411,** 17–19.

Snyder A.M. (2000) *The Effects of Elevated Carbon Dioxide and Temperature on two Cultivars of Rice*. Master of Science Thesis. University of Florida, Gainesville, FL, USA.

Stott P.A., Tett S.F.B., Jones G.S., Allen M.R., Mitchell J.F.B. & Jenkins G.L. (2000) External control of 20th century temperature by natural and anthropogenic forcing. *Science* **290,** 2133–2137.

Suzuki Y., Makino A. & Mae T. (2001) Changes in the turnover of Rubisco and levels of mRNAs of *rbcL* and *rbcS* in rice leaves from emergence to senescence. *Plant, Cell and Environment* **24,** 1353–1360.

Tyysjärvi E., Mäenpää P. & Aro E.-M. (1994) Mathematical modelling of photoinhibition and photosystem II repair cycle. I. Photoinhibition and D1 protein degradation *in vitro* and in the absence of chloroplast protein synthesis *in vivo*. *Photosynthesis Research* **41,** 439–449.

Vu J.C.V., Allen L.H. Jr, Boote K.J. & Bowes G. (1997) Effects of elevated CO_2 and temperature on photosynthesis and Rubisco in rice and soybean. *Plant, Cell and Environment* **20,** 68–76.

Vu J.C.V., Gesch R.W., Pennanen A.H., Allen L.H. Jr, Boote K.J. & Bowes G. (2001) Soybean photosynthesis, Rubisco, and carbohydrate enzymes function at supraoptimal temperatures in elevated CO_2. *Journal of Plant Physiology* **158,** 295–307.

Webber A.N., Nie G.-Y. & Long S.P. (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. *Photosynthesis Research* **39,** 413–425.

Xie Y. & Wu R. (1988) Nucleotide sequence of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene (*rbcS*) in rice. *Nucleic Acids Research* **16,** 7749.

Yoshida S. (1981) *Fundamentals of Rice Crop Science.* pp. 65–67. International Rice Research Institute, Los Baños, Philippines.

Ziska L.H., Manalo P.A. & Ordonez R.A. (1996) Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO_2 and temperature: growth and yield response of 17 cultivars. *Journal of Experimental Botany* **47,** 1–7.

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